4 290764

(12) UK Patent Application (19) GB (11) 2 368 904 (13) A

(43) Date of A Publication 15.05.2002

(21) Application No 0027523.0

(22) Date of Filing 10.11.2000

(71) Applicant(s)
Zetatronics Limited
(Incorporated in the United Kingdom)
Department of Biosciences,
University of Hertfordshire, Hatfield Campus,
College Lane, HATFIELD, Herts, AL10 9AB,
United Kingdom

(72) Inventor(s)
Stuart Harbron

(74) Agent and/or Address for Service
Sommerville & Rushton
45 Grosvenor Road, ST ALBANS, Herts., AL1 3AW,
United Kingdom

(51) INT CL[7]
G01N 27/447

(52) UK CL (Edition T)
G1A AA3 AEXX AG17 AG18 AR7 AT2 AT22 AT3

(56) Documents Cited
GB 2335981 A
US 4101220 A
WO 88/02482 A1
US 3870612 A

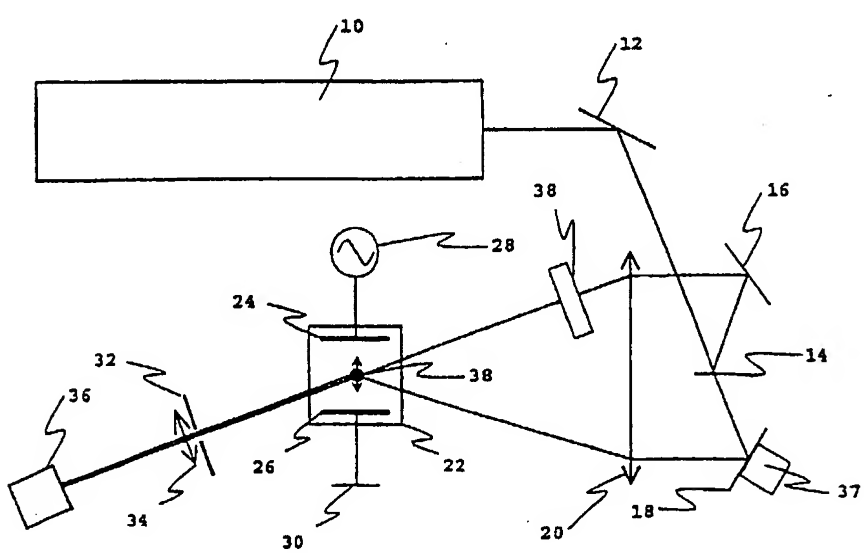
(58) Field of Search
UK CL (Edition S) G1A AAJX AEDX AEG AEH AEJX AEXX , H4D DLAB DLVD DLVX
INT CL[7] G01N 27/447
Online: WPI, EPODOC, JAPIO

(54) Abstract Title

Obtaining information about zeta potential, electrophoretic mobility and numbers of suspended particles

(57) An apparatus for obtaining information about the zeta potential, electrophoretic mobility and/or numbers of one or more particles 38 dispersed in a fluid comprises means 22, 24, 28 for establishing an electric field across a said dispersion of particles in a fluid to move the particles through the fluid at a velocity proportional to their electrophoretic mobilities in a direction parallel with the electric field; laser means 10 for generating a light beam of monochromatic or quasi-monochromatic electromagnetic radiation incident on the particles so that the light beam is scattered by the particles; and detector means 36 for detecting the light scattered by the particles whereby the zeta potential, electrophoretic mobility and/or numbers of the particles in the fluid may be determined, wherein the means for establishing an electric field is adapted to apply an oscillating electric field to the fluid.

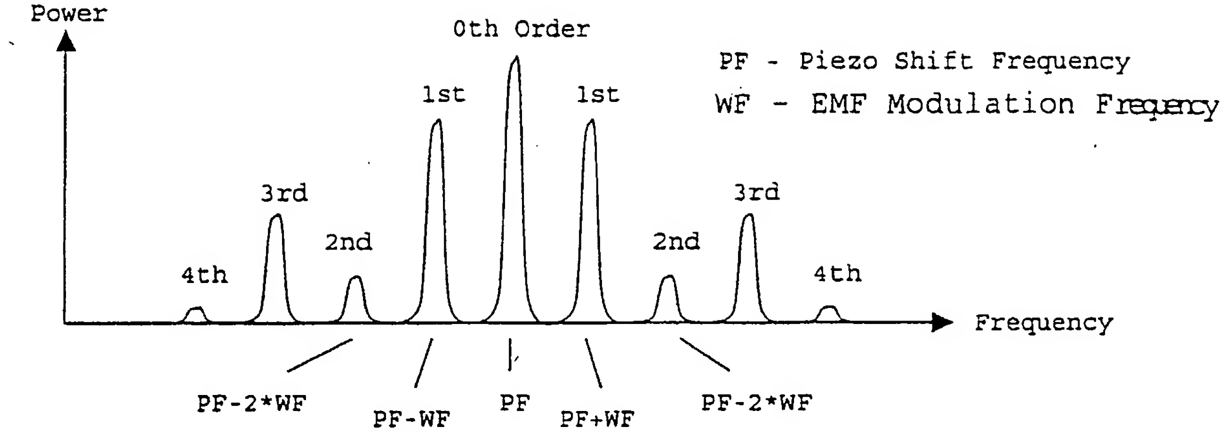
Figure 1



At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.
The claims were filed later than the filing date but within the period prescribed by Rule 25(1) of the Patents Rules 1995.
This print takes account of replacement documents submitted after the date of filing to enable the application to comply

GB 2 368 904 A

Figure 2


Power
0th Order
PF - Piezo Shift Frequency
WF - EMF Modulation Frequency
1st
1st
3rd
3rd
2nd
2nd
4th
4th
Frequency
PF-2*WF
PF-WF
PF
PF+WF
PF-2*WF

velocity spectrum. By measuring the magnitudes of the peaks in a Fourier power spectrum of the scattered light while applying an oscillating field, the present inventors are able to overcome or mitigate these shortcomings

Summary of the Invention

The inventors have discovered that applying an oscillating electric field to a sample containing particles, the amplitude and relative phases of the peaks in a Fourier power spectrum of the scattered light as a function of either the applied frequency or voltage is unaffected by confounding factors such as Brownian movement and fluid turbulence.

They have further discovered that the amplitude of the n'th order peak has been determined to be proportional to the n'th order Bessel Function as a linear function of the particle zeta potential or electrophoretic mobility divided by the EMF modulation frequency, and the total power is a function of the scattering efficiency, which is dependent on the number of particles present. The relative phases of odd and even n'th order peaks are determined by the direction of the particle velocity and an initial random phase constant.

In general terms, one aspect of the present invention is an apparatus for obtaining information about the zeta potential or electrophoretic mobility and numbers of one or more particles dispersed in a fluid. In one embodiment the apparatus comprises a laser Doppler anemometer, in which an oscillating electric field is applied across the region of the sample where the beams of laser light are made to cross, a detector, and an analyser. The laser Doppler anemometer (LDA) may be a reference beam LDA or a cross beam LDA.

by means of a detector; transforming said signal to yield a Fourier power spectrum; calculating the power and relative phase of each peak in the power spectrum; and converting this information into zeta potential or electrophoretic mobility.

It is an object of the invention to provide an apparatus able to distinguish electrophoretic movement from Brownian motion, thermal turbulence and motion due to flow or electrosmosis.

Detailed Description of the Invention

Referring now to Figure 1, one embodiment of the present invention is disclosed in diagrammatic form. A reference beam LDA system, as shown in figure 1, is used to measure the zeta potential or electrophoretic mobility statistics of a fluid cell containing particles that are placed in an oscillating electromagnetic field.

The embodiment shown in Figure 1 comprises a quasi-monochromatic light source **10** producing a beam of collimated light. The light source may be a laser, and is preferably a Helium-Neon gas laser or a laser diode. The beam is made to impinge on a first mirror **12** and is reflected onto beam splitter **14** to produce a second beam and a third beam. Mirror **12** permits easier alignment of the apparatus; in an alternative configuration the beam is made to impinge directly on beam splitter **14**. The second beam is reflected onto a second mirror **16** and passes through an attenuator **38** to produce a reference beam. The third beam is reflected onto a third mirror **18**. The third mirror is attached to a vibrating unit **37**, preferably a piezo-electric element, so as to produce a phase-shift in the light beam. The vibrating unit operates at a frequency of between 100 Hz and 3 kHz, preferably at 250 Hz to 1 kHz. Most preferably the vibrating unit follows a ramp or saw-tooth oscillation having an amplitude of one wavelength, which for the preferred helium-neon laser is

Theory

The movement $\varphi(t)$ of a single particle in the system with time t can be expressed mathematically as the following differential equation:

acceleration force + stokes drag = driving force

$$m_p \frac{d^2\varphi}{dt^2} + 3\pi\eta d_p \frac{d\varphi}{dt} = kV_p \sin \omega t$$

where m_p is the particle mass, η is viscosity, d_p the particle diameter, the product kV_p the maximum force on the particle (linear with maximum or peak to peak EMF V_p) and ω the frequency at which the force oscillates.

This equation has the following solution,

$$\varphi(t) = \frac{kV_p}{2j}\left[\left(\frac{1}{3\pi\eta d_p j\omega - m_p\omega^2}\right)\exp(j\omega t) + \left(\frac{1}{3\pi\eta d_p j\omega + m_p\omega^2}\right)\exp(-j\omega t)\right]$$

For particles being moved under low frequency EMF, $|3\pi j\eta d_p| >> m_p\omega$, thus

$$\varphi(t) = \frac{-kV_p}{3\pi\eta d_p\omega}\cos(\omega t)$$

Inside the reference beam LDA system, the particle scatters light such that the electric field at the photodiode is,

$$\begin{aligned} U(t) &= \text{reference beam} + \text{particle scatter beam} \\ &= A_R \exp(j[\phi_0 - \psi t]) + A_P \exp\left(\frac{2\pi j}{\lambda_f}\varphi(t)\right) \end{aligned}$$

Simplifying to,

$$\mathrm{FT}\{I(t)\} = \exp(j\phi_0)\sum_{m=-\infty}^{\infty}\exp\left(2\pi jm\left[\frac{f-\psi}{\omega}\right]\right)\int_{\tau=0}^{2\pi}\exp\left(j\left[\left(\frac{f-\psi}{\omega}\right)\tau-\frac{\kappa V_p}{\omega}\cos(\tau)\right]\right)d\tau$$
$$+\exp(-j\phi_0)\sum_{n=-\infty}^{\infty}\exp\left(2\pi jn\left[\frac{f+\psi}{\omega}\right]\right)\int_{\tau=0}^{2\pi}\exp\left(j\left[\left(\frac{f+\psi}{\omega}\right)\tau+\frac{\kappa V_p}{\omega}\cos(\tau)\right]\right)d\tau$$

Considering the two infinite series, these are only convergent if and only if the exponential phase term is either stationary or an integer multiple of 2π. For this to be true for the first summation,

$$f = \psi + m\omega$$

and for the second series,

$$f = -\psi + n\omega$$

Also the two integrals can be evaluated using the following standard Bessel integral:

$$J_\alpha(\beta) = \frac{1}{2\pi j^\alpha}\int_0^{2\pi}\exp(j[n\chi + \beta\cos\chi])\,d\chi$$

Thus,

$$\mathrm{FT}\{I(t)\} = 2\pi\exp(j\phi_0)\sum_{m=-\infty}^{\infty}\{j^m J_m(-\kappa V_p/\omega)\,\delta(f-\psi-m\omega)\}$$
$$+2\pi\exp(-j\phi_0)\sum_{n=-\infty}^{\infty}\{j^n J_n(\kappa V_p/\omega)\,\delta(f+\psi-n\omega)\}$$

That is the Fourier Transform of the intensity measured by the detector is simply a comb function, the m'th peak away from the zero'th order at $f=\psi$ having an amplitude defined by the m'th order Bessel Function of the first kind as a function of ω, κ and V_p.

Therefore, by measuring the relative heights and phases of the peaks obtained from Fourier Transforming the voltage signal, the magnitude of the zeta potential or

Claims

1. An apparatus for obtaining information about the zeta potential, electrophoretic mobility and/or numbers of one or more particles dispersed in a fluid, the apparatus comprising:

means for establishing an electric field across a said dispersion of particles in a fluid to move the particles through the fluid at a velocity proportional to their electrophoretic mobilities in a direction parallel with the electric field;

laser means for generating a light beam of monochromatic or quasi-monochromatic electromagnetic radiation incident on the particles so that the light beam is scattered by the particles;

and detector means for detecting the light scattered by the particles whereby the zeta potential, electrophoretic mobility and/or numbers of the particles in the fluid may be determined,

wherein the means for establishing an electric field is adapted to apply an oscillating electric field to the fluid.

2. An apparatus as claimed in claim 1, wherein the detector means generates an electrical signal corresponding to the detected scattered light and has an analyser therein or linked thereto which is programmed to process the signal from the detector to yield a Fourier power spectrum.

3. An apparatus as claimed in claim 2, wherein the analyser is further programmed to calculate the power and relative phase of one or more peaks in the power spectrum.

providing an apparatus as claimed in any preceding claim and applying an oscillating electric field across a measuring region of the fluid and detecting light scattered in one or more predetermined directions by the one or more particles moving within the electric field; and analysing the scattered light.

11. A method as claimed in claim 10, which further comprises the step of analysing the detected scattered light using a Fourier power spectrum.

12. A method as claimed in claim 10 or 11, wherein the detected scattered light is converted to an electrical signal by the detector and this signal is transformed to yield a Fourier power spectrum and the power and relative phase of at least one peak in the power spectrum is calculated to determine the zeta potential or electrophoretic mobility of one or more particles dispersed in a fluid being measured.

13. A method as claimed in claim 12 wherein the power spectrum is analysed using a Bessel function.

14. An analyser for use in the apparatus of claim 1, the detector means of the apparatus of claim 1 generating an electrical signal corresponding to detected scattered light and the analyser being programmed to process the signal from the detector to yield a Fourier power spectrum.

15. An analyser as claimed in claim 14, wherein the analyser is further programmed to calculate the power and relative phase of one or more peaks in the power spectrum.

14

Application No: GB 0027523.0 **Examiner:** Bob Clark
Claims searched: All **Date of search:** 24 September 2001

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S): G1A (AAJX, AEG, AEH, AEDX, AEJX, AEXX); H4D (DLAB, DLVD, DLVX)

Int Cl (Ed.7): G01N 27/447

Other: Online: EPODOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
Y	GB 2335981 A	(ZETATRONICS) Line 22 p.12 to line 11 p.14	5-7
A	WO 88/02482 A1	(O'BRIEN)	
X, Y	US 4101220	(BEAN et al.) Line 50 column 2 to line 23 column 3	X:1-3,8-15 Y:5-7
A	US 3870612	(FLYGARE et al.)	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
		E	Patent document published on or after, but with priority date earlier than, the filing date of this application.
&	Member of the same patent family		

16. An analyser as claimed in claim 15, wherein the analyser is programmed to analyse the power spectrum using a Bessel function.

4. An apparatus as claimed in claim 2 or claim 3 wherein the analyser is programmed to analyse the Fourier power spectrum using a Bessel function.

5. An apparatus as claimed in any of claims 1 to 4, wherein the apparatus further comprises beam splitter means to produce a second and third beam from the light beam, and beam focusing means to focus the second and third beam to intersect within the electric field.

6. An apparatus as claimed in claim 3, wherein at least one of the second and third beams is altered in frequency or phase so that it is different from the other.

7. An apparatus as claimed in claim 5 or 6 wherein the second or third beam is attenuated to form a reference beam and the detector is arranged to detect the intensity of the light scattered from the particles and interfered with said reference beam.

8. An apparatus as claimed in claim 1 wherein the apparatus comprises a reference beam Laser Doppler Anemometer or a crossbeam Laser Doppler Anemometer.

9. An apparatus as claimed in any preceding claim in combination with a sample vessel holding one or more particles dispersed in a fluid, the sample vessel being positioned within the electric field and the light beam passing therethrough.

10. A method for obtaining information about the zeta potential or electrophoretic mobility of one or more particles dispersed in a fluid, which method comprises:

electrophoretic mobility of the particle can be derived since κ is a linear function of these two constants.

The foregoing has described one embodiment of the present invention. In another embodiment, the beam of quasi-monochromatic coherent light may be split into two beams having different frequencies and different relative phases by, for example causing it to impinge on a rotating diffraction grating. Different optical layouts may be used to achieve the same end, namely to cross two beams differing in temporal and spatial frequencies in a region of the fluid cell between the two electrodes, and detect the scattered light. The modulated laser Doppler signal may be measured along an attenuated reference beam (the heterodyne mode) as shown in Figure 1, or the light scattered may be measured (homodyne mode).

The fluid cell shown in Figure 1 has one electrode at earth potential; in another embodiment positive voltage is applied to one electrode and a negative voltage is applied to the other.

where A_R and A_P are the magnitudes of the light from the reference and beam scattered by the particle, λ_f is the fringe spacing, and ψ is the frequency shift, as for example provided by the piezoelectric mirror.

The intensity at the detector is then,

$$\begin{aligned} I(t) &= U(t)U^*(t) \\ &= A_R{}^2 + A_P{}^2 + 2A_R A_P \cos\left(\phi_0 - \psi t - \frac{2\pi}{\lambda_f}\varphi(t)\right) \end{aligned}$$

That is,

$$I(t) = K_0 + K_1 \cos\left(\phi_0 - \psi t - \frac{\kappa V_p}{\omega}\cos(\omega t)\right)$$

where $\kappa = -\frac{2k}{3\lambda_f \eta d_p}$. This constant is a linear function of the zeta potential or electrophoretic mobility of the particle.

Now consider Fourier Transform of the intensity measured by the detector,

$$FT\{I(t)\} = \int_{-\infty}^{\infty} I(t)\exp(jft)dt$$

$$\begin{aligned} FT\{I(t)\} \propto & \int_{-\infty}^{\infty} \exp\left(j\left[\phi_0 - \psi t - \frac{\kappa V_p}{\omega}\cos(\omega t)\right]\right)\exp(jft)dt \\ & + \int_{-\infty}^{\infty} \exp\left(-j\left[\phi_0 - \psi t - \frac{\kappa V_p}{\omega}\cos(\omega t)\right]\right)\exp(jft)dt \end{aligned}$$

Evaluating the first integral by making the substitution $\tau = \omega t$ and splitting the integral into a series of smaller definite integrals,

$$\begin{aligned} FT\{I(t)\} = & \exp(j\phi_0)\sum_{m=-\infty}^{\infty}\int_{\tau=0}^{2\pi} \exp\left(j\left[\left(\frac{f-\psi}{\omega}\right)(\tau + 2\pi m) - \frac{\kappa V_p}{\omega}\cos(\tau + 2\pi m)\right]\right)d\tau \\ & + \exp(-j\phi_0)\sum_{n=-\infty}^{\infty}\int_{\tau=0}^{2\pi} \exp\left(j\left[\left(\frac{f+\psi}{\omega}\right)(\tau + 2\pi n) + \frac{\kappa V_p}{\omega}\cos(\tau + 2\pi n)\right]\right)d\tau \end{aligned}$$

652nm. Light beams reflected from the second and third mirrors are focused by a lens **20** and intersect in the centre region of a measurement cell **22**. The cell comprises two electrodes **24** and **26**, which are connected respectively to an alternating voltage source **28** and earth **30**. Light scattered from particles **38** in the sample contained by the cell is interfered with the reference beam and passes through aperture **32** and lens **34**, and onto detector **36**. The detector may be a photodiode or photo-multiplier, and is preferably a silicon photodiode.

The speckle intensity of the scattered light is measured as a function of time. The Fast Fourier Transform of this signal is averaged over time to remove speckle fluctuation. Preferably between 10 and 200 transforms are averaged, most preferably about 100 transforms are averaged. Referring now to Figure 2, the average Fast Fourier Transform of the voltage signal produced by the detector produces a comb frequency spectrum centred on a zero order peak originating from the mirror vibrating unit. The separation of each spike in the transform shown in Figure 2 is determined by the frequency of the alternating voltage source oscillations. Random movement due to Brownian motion and systematic errors arising from electro-osmosis induced fluid turbulence broaden these peaks but does not affect their total magnitudes/power.

The inventors have determined that by classifying according to the amplitude and relative phases of the peaks in the Fourier Power Spectrum as a function of either the frequency or voltage applied, the zeta potential or electrophoretic mobility and numbers of one or more species of particle can be accurately determined. Since the magnitudes of the peaks are unaffected by confounding factors such as Brownian movement and fluid turbulence, measurements made using this technique will also effectively be independent of such confounding influences.

In a preferred embodiment, the apparatus comprises a vessel for holding the fluid in which the particle or particles to be analysed are dispersed; a quasi-monochromatic light source; a means for producing a second and a third coherently-related light beam from a first beam produced by said light source; a phase-shifting means to shift the phase of said second or third light beam; a means to apply an oscillating electric field across the measurement region of the fluid; a focussing means to cause said two or more beams to cross at a predetermined point in said measurement region; detection means to detect light scattered by said particle or particle, said detection means arranged to detect scattered light in one or more predetermined directions; and means to analyse light scattered by said particle or particles.

In a particularly preferred embodiment, the apparatus additionally comprises a means to reduce the intensity of either said second or third light beam to form a reference beam, and the detection means is arranged to detect the intensity of the light scattered from the particles and interfered with said reference beam.

Another aspect of the invention is a method for obtaining information about the zeta potential or electrophoretic mobility of one or more particles dispersed in a fluid. The method comprises applying an oscillating electric field across a measuring region of the fluid contained in the sample container of a laser Doppler anemometer; detecting light scattered in one or more predetermined directions by said one or more particles moving between said electrodes; and analysing said scattered light.

Another aspect of the invention is a method for analysing light scattered in a predetermined direction by one or more particles moving in an oscillating electric field. The method comprises converting the scattered light into an electrical signal

IMPROVED APPARATUS

Background art

Laser Doppler anemometry (LDA) has been applied to the measurement of electrophersis of particles dispersed in solution. For example, Flygare and Ware (U.S. Pat. No. 3,870,612) disclose an electrophoretic scattering apparatus for determining the electrophoretic mobility and diffusion coefficient of a macromolecular polymer in solution is disclosed. More particularly, the charged macromolecules are driven through the solution by a constant electric field developed between two charged electrodes in a modified electrophoretic cell. The electric field is alternately enabled and disabled during the determination to prevent excessive heat build-up in the solution and the resultant convection of macromolecules, which would distort the measurements. A laser provides an incident light beam which is passed through the cell perpendicular to the direction of the macromolecule flow so that the autocorrelation function or, alternatively, the frequency spectrum of the light scattered from the macromolecules can be observed at low scattering angles. The scattered light, in addition to having a frequency distribution curve proportional to the diffusion coefficient of the macromolecules, is Doppler shifted by an amount proportional to the electrophoretic mobility of the macromolecule in the scattering region. Because each species of polymers has a unique electrophoretic mobility and hence Doppler shift, the apparatus is useful in quantitatively analysing a mixture of several different polymers in solution.

These systems suffer from a number of drawbacks. First, thermal currents and electro-osmosis causes the fluid to drift within the cell during the measurement process, which causes peak broadening. Secondly Brownian motion broadens the

Figure 1